

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Appl. No. : **09/328,975**
Applicants : **Jon A. Wolff, et al.**
Filed : **06/09/1999**
Art Unit : **1635**
Examiner : **Schnizer, Richard**
Docket No. : **Mirus009**
For: **Charge Reversal of Polyion Complexes**

Confirmation No. 7574

Commissioner of Patents
PO Box 1450
Alexandria, VA 2231-1450

DECLARATION UNDER 37 C.F.R. ' 1.132

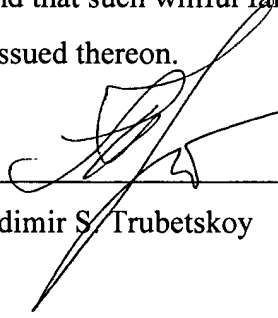
Dear Examiner:

I, Vladimir Trubetskoy, hereby declare as follows:

1. I am an inventor of the captioned application.
2. The attached notebook pages (nos. 120-122) contain the experiment and data that is described in the specification in example 6 starting on page 26.
3. The abbreviation used throughout this notebook for polyaspartic acid was pAsp (see page 22 of the notebook). The abbreviation for polyacrylic acid used throughout the notebook was pAA (see page 111 of the notebook).
4. The polymer used in the experiment disclosed in the specification in example 6 was polyacrylic acid.
5. The abbreviation listed in the specification on page 22 line 27 for polyaspartic acid is a typographical error. The correct abbreviation for polyaspartic acid is found on page 6 line 26 and page 20 line 4 of the specification. Also note that the abbreviation pMAA, for

polymethylacrylic acid (see page 6 line 26 and page 20 line 4 and page 111 of the notebook), is consistent with PAA being the abbreviation for polyacrylic acid.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.



Dr. Vladimir S. Trubetskoy

4/18/05
Date

VIAL	43	237	LR	> Liver
#3	44	208	MC	
SpLL	45	311	Spleen	
	46	151	Lung	
	47	128	heart	
	48	184	Kidney	
	49	346	tail	
SAMPLE		RLU		

VIAL	1	221	LR	> Liver
#3	2	234	MC	
SpLL	3	173	Spleen	
	4	156	Lung	
	5	141	heart	
	6	156	Kidney	
	7	395	tail	

VIAL	8	216	LR	
#4	9	142	MC	
	10	163	Spleen	
PEG XL	11	187	Lung	
	12	177	heart	
	13	413	Kidney	
	14	221	tail	
	15	185	LR	> LIVER
VIAL	16	198	MC	
#4	17	161	Spleen	
PEG XL	18	162	heart	
	19	217	Kidney	
	20	206	Kidney	
	21	217	Tail	

In general, though all numbers are very low non XL samples worked better (liver 15 times, tail)
So XL-particles are too stable

DNA/PLL34/PEG-SPLC particles did not aggregate upon addition of salt. results in highest tail activity

2/10/99

Synthesis of PEG-pAsp conjugates.

Polyspartic acid (pAsp) $M_w = 36,000$ - decondensing polyanion

Idea - to modify pAsp with increasing # of PEG chains to modify its ability to decondense DNA from its DNA/PLL complex

% of modification will establish the rate of DNA release

V. Trubetsky 2.10.99

Pl-DNA decondensation with different polyacrylates

Polyacrylates: pMAA 9 μ Dg
pAA 30 μ Dg
pPAA 8 μ Dg Daves

DNA* D_g was condensed with 12 & PLL 34
in 5 mM HEPES pH 7.5.

No.	FI	[ug/ml]	10x of each polyacrylate were added to decondense
1	-0.016	0.0118	
2	56.588	0.4026 DNA	
3	8.452	0.0703 DNA/PLL	
4	46.071	0.3300 10x pAA	
5	56.180	0.3998	
6			
7			
8			
9			
10			
11			
12			
13			

No.	FI	[ug/ml]	
1	-0.028	0.0118	
2	53.121	0.3787 DNA	
3	8.696	0.0720 DNA/PLL 34	
4	54.085	0.3854 10x pPAA	
6	48.614	0.3476 DNA	
7	8.687	0.0719 DNA/PLL	
8	9.190	0.0754 10	
9	8.740	0.0723 20	
10	8.083	0.0678 30	
11	7.983	0.0671 40	
12	7.660	0.0648 50	
13	7.405	0.0631 60	

but does not work

4.29 ~~99~~ Cell-binding results of experiment on p. 110.

Polyanions alone did not work - no all bind,
but probably concentration was too low

Particles

(1) SP0210 vs SP0460 no XL - particles definitely disassemble on cell surface, SP0 almost invisible only DNA (may be intensity), no internalization

460 particles are more stable than 210 (note colocalization)

(2) XL particles bind less than no-XL but with more colocalization (expectable)

(3) noXL-460 one can see some SP0 binding

1/2 in vivo DNA/PEI/pAA

Samples recharged ~ 4L pAA before and after
neutrality DNA/PEI/pAA

per animal: (1) 50g / 100g / 40g
(2) — 11 — 50g +
(3) — 11 — 60g + } cloudy in 0.25 ml
(4) — 11 — 70g } 1.6

Results of in vitro binding exp. from So and Kirk

BNL
Hela

Kirk: all polyanions binds very weakly more with
RGD less with PEG and SPL

Particles bind more strongly with RGD more
PEG less much more with SPL

So: General binding less than usual (XL!)
with no difference from amount of SPL

5/6/99

Work with cyclic RGD peptide

RGD peptide (Mirus) GGCRGDMFGC $M_w = 1000$

18 mg diss. in 1 ml MeOH + 15X of conc. HCl

add 40 μ l of I_2 solution in MeOH (50%)

— in 5X increments \rightarrow until yellow color stays

Precipitate with $2H_2O$

8 mg of peptide + 27.2 mg of Fmoc-PEG-NHS

~~conc.~~ in 0.7 ml of 0.1M HEPES pH 8.0, 0.5M $NaCl$

WAVE

disap. of M_{12} groups during reaction

S# 420.0

1 0.0003

2 0.0060 after

3 0.6759 before react

10X / 0.5 ml of borax

after reaction - dial. of K_2O in 3.5 MDS
MWCO overnight 4°C.

folate particles for So:

percent from p 114.

- (1) Rh DNA/PCL34 / C₆SPLC210 - PEG folate 50/70/250
 (2) — 11 — SPLC(51.)-PEG — 11 —
 (3) — 11 — C₆SPLC210 50/70/150

activated with 100/200 SDC/5-mus

Results of 1/2 low pressure injections from p 120

#1			#2		
	1	1548 LR	19	3354 LR	
	2	1211 MC	20	6594 MC	Vial 2
VIAL	3	1093 Spleen	21	2461 Spleen	(210)
100	4	166931 lung	22	10074 lung	Animal 5
Animal	5	2722 Heart	23	211 Heart	
1	6	381 Kid.	24	136 Kidney	50/100/50
	7	4041 LR	25	1374 LR	
VIAL	8	1986 MC	26	2538 MC	
100	9	6412 Spleen	27	2293 Spleen	Animal 6
Animal	10	197443 lung	28	16544 lung	Vial 2
2	11	1649 Heart	29	196 Heart	(210)
50/100/40	12	916 Kidney	30	178 Kidney	

one animal died 1/3

#2			one animal died (1/4)		
VIAL	13	2951 LR			
2 (210)	14	2290 MC			
Animal	15	1022 Spleen			
100	16	1557 lung			
	17	66 Heart			
	18	12144 Kidney			

31 11604 LR ^{#3}
 32 5822 MC 50/100/60
 33 225 Spleen Vial 3
 34 333 lung 7 Animal
 35 76 Heart
 36 91 kidney
 37 21517 LR
 38 22765 MC Vial 3
 39 4404 Spleen Animal #9
 40 290 lung
 41 57 Heart
 42 82 kidney
 43 12606 LR
 44 12913 MC Vial 3 (BKC)
 45 314 Spleen Animal #9
 46 352 lung
 47 95 Heart
 48 67 kidney

Surv.

(0/3)

49 254 LR ^{#4}
 50 147 MC 50/100/70
 51 556 Spleen
 52 70368 lung
 53 583 Heart
 54 139 kidney
 55 388 LR
 56 587 MC
 57 1507 Spleen
 58 150514 lung
 59 186 Heart
 60 260 Spleen
 61 429 LR
 62 407 MC
 63 1858 Spleen
 64 266123 lung
 65 3018 Heart
 66 288 kidney

Surv.

(0/3)

Vial
 #4
 Animal
 #10

Animal
 #11

Animal
 #12